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Full Length Research

Antioxidant and Micronutrient Status of Nigerian Males Occupationally Exposed to Lead

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All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA. A total of 150 participants consisting of 50 battery chargers (BC), 50 auto painters (AP) and 50 office workers (controls) were selected for the study. Lead (blood lead levels-BLL), Zinc (Zn), Iron (Fe) and Selenium (Se) levels and were determined in the whole blood and serum using the Atomic Absorption Spectrophotometer (AAS). Glutathione Peroxidase (GPx), Catalase (CAT), Superoxide Dismutase (SOD), Total Plasma Peroxide (TPP) and other antioxidants were also determined in the serum using spectrophotometric techniques. Oxidative Stress Index (OSI) was computed. The BLL, TPP, and OSI were all significantly elevated in lead workers ($p<0.0001$ in all cases). In contrast the micronutrients, Fe, Se, and Zn were all significantly reduced in lead workers ($p<0.0001$). The GPx, CAT, SOD, Uric acid and Vitamin C levels were all significantly reduced in lead workers ($p<0.0001$; 0.01(for uric acid). Lead suppresses the micronutrient and antioxidant status to induce oxidative stress in exposure.

KEY WORDS: Lead, battery chargers (BC), auto painters (AP), Oxidative Stress Index (OSI).

INTRODUCTION

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and a biological systems ability to readily detoxify the reactive intermediates or easily repair the

resulting damage (Valko et al., 2007). Oxidative stress has been implicated in numerous disease states such as cancer, connective tissue disorders, aging, infection, inflammation, AIDS and male infertility (Clark et al., 1986; Aitken and Krausz, 2001). Studies have shown that lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydyl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities and increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition (Gurer and Ercal, 2002).

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (Yadav et al., 2016). In a normal cell, there is an appropriate pro-oxidant: antioxidant balance. Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as pro-oxidants. On the other hand, compound and reactions disposing of these species, scavenging them, suppressing their formation, or opposing their actions are antioxidants (Anu et al., 2014).

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radicals intermediates, and inhibit other oxidation reactions by being oxidized themselves. However, this balance can be shifted toward the pro-oxidants when production of oxygen species is increased greatly as seen in the ingestion of certain chemicals or drugs or when levels of antioxidants are diminished as seen by inactivation of enzymes involved in disposal of oxygen species. This state called oxidative stress can result in serious cell damage if the stress is massive or prolonged. (Murray, 2005).

Antioxidant defense mechanisms include three levels of protection as prevention, interception and repair (Sies, 2007). Prevention of ROS formation is the first line of defense against oxidative insult. An example is the binding of metal ions, iron, and copper ions in particular which prevents them from initiating a chain reaction (Sies, 2007). Chelation of transition metals is a major means of controlling sperm lipid peroxidation and DNA damage. When transition metals become loosely bound to oxygen reduction products, they can produce secondary and more reactive oxidants, particularly the hydroxyl radical (OH) (Aitken et al., 2014). During interception, free radicals have a tendency toward chain reaction (compound carrying an unpaired election reacts with another compound to generate another unpaired electron, “radical begets radical”. Hence, the basic problem is to break this chain reaction by formation of non-radical end products (Sies, 2007).

Battery chargers and painters are among the various workers who are constantly exposed to lead. Lead salts form the basis of many paints and pigments (Royal Society of Chemistry, 2007). Pigments, lead (II) chromate ($PbCr0_4$ “chrome yellow”) and lead (II) carbonate ($PbC0_3$ “white lead”) are the most common forms (Royal Society of Chemistry, 2007). Lead is added to paint to speed up drying, increase durability, maintain a fresh appearance and resist moisture that causes corrosion. In some countries like Nigeria, lead continues to be added to paint, whereas countries such as United States and United Kingdom have regulations prohibiting this (Anne, 2009). The largest single use of lead is for the manufacture of accumulator (lead combined with sulphuric acid to generate voltage in excess of 2 volts for starting cars) (Richards, 2009). This industry used both metallic lead in the form of lead antimony alloy, and lead oxides in about equal proportions. The metallic lead is in the grid and lugs while the oxides litharge (PbO) are used in the active material that is pasted on the plates. In lead acid battery charging, the mixing of lead oxide paste runs parallel to grid casting. Here the major hazard is from the lead oxide dust, particularly when loading the mixer with lead oxide powder.

In May 2009, the United Nations Panel on Environmental Disaster (UNPED), called for the formation of a worldwide partnership to ban the use of lead in paint (International Strategy for Disaster Reduction, 2009). According to the panel, people around the world especially children are at risk of lead poisoning from battery recycling operations, smelting plants and other sources including lead

paint. The ban according to UNPED will at least encourage countries to change their laws so that the public will become aware and be concerned about it (WHO, 2009). This call seemed not to have made any impact as so many people are still constantly being exposed to lead through occupational exposure particularly in developing countries of the world.

MATERIALS AND METHODS

STUDY SITE

Surulere is a residential, commercial and a local government area located on the Lagos mainland in Lagos State with an area of 23km^2. It is a part of metropolitan Lagos. At the last census in the year 2006, there were 502, 865 inhabitants, with a population density of 21, 864 inhabitants per square kilometer. It is inhabited mostly by the Yorubas and the Igbos. It has a General hospital at Randle Street, 3 Primary Health Care Centres and more than One Thousand Private Hospitals and other Health Care facilities.

METHODOLOGY

This was a cross sectional study carried over six months. A total of 100 lead exposed male workers comprising 50 lead acid battery chargers and 50 auto painters within Surulere LGA of Lagos State were used in this study. Subjects were all Nigerians within the age bracket of 18 and 60 years randomly selected. The Ethical approval was obtained from the Institutional Review Board of the Nigerian Institute for Medical Research, Lagos State. Informed consent of the subjects was gotten through the aid of a well-structured questionnaire. Verbal presentations of the summaries of the hazards of lead exposure were made to the sample population to encourage voluntary participation of the subjects. Inclusion criteria were all participants in the study and it was purely voluntary. We excluded from the study those on any systemic diseases, those on fertility drugs, those who smoke, all those with history of drugs/substance abuse, all women and those on immune suppressive drugs. The control samples comprised of 50 males office workers who had never been occupationally exposed to lead.

Sample Collection

Blood

Ten milliliters of venous blood were collected from each subject. The samples were divided into three equal parts in lead-free heparinized tubes, EDTA bottles and plain bottles. The samples were collected in the morning between 8 am and 10 am and those for lead were done within three hours of collection. Samples in the plain bottles were allowed to clot, centrifuged, separated and the serum stored at -4^{o}C until analyzed.

LABORATORY ANALYSIS

Determination of Lead, Zinc, Selenium and Iron

Blood lead levels (BLL) were determined in the whole blood while Zn, Se and Fe were determined in the serum using the Atomic absorption spectrophotometer (AAS).
Principle: The energy absorbed by atoms excited from the ground state is characteristic of those atoms. If light with the same wavelength characteristics as the radiation absorption of the element to be analysed is passed through a curtain of flame containing those atoms in the excited state, the amount of light energy absorbed will be proportional to the concentration of the element.

Determination of Vitamin C

Principle: Ascorbic acid in serum is oxidized by Cu (II) to form dehydroascorbic acid, which reacts with acidic 2, 4 – dinitrophenylhydrazine to form a reddish – hydrazone, which was measured spectrophotometrically at 520 nm.

Determination of Catalase Activity (INDIRECT METHOD)

Principle: This was based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchromic acid as an unstable intermediate. The chromic acetate produced is measured spectrophotometrically between 570 - 610 nm. The

catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction was stopped at a particular time (10 minutes) by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 was determined by measuring chromic acetate after heating the reaction mixture.

Determination of Superoxide Dismutase (SOD)

Super Oxide Dismutase estimation was carried out by colorimetric method using a commercially prepared reagent manufactured by Randox laboratories Ltd, Antrim, UK. The principle was based on the fact that xanthine oxidase generates superoxide radicals which react with 2 (4-iodophenyl)-3-(nitrophenol)-5-phenyltetrasodium chloride (INT) to form a red formation dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction. One unit of SOD caused a 50% inhibition of the rate of reduction of INT under the condition of the assay.

Determination of Total Plasma Peroxide (TPP)

Principle: Ferrous-butylated hydroxytoluene-xylenol orange reacts with plasma hydrogen peroxide to form a colour complex measured spectrophotometrically at 560 nm. H_2O_2 was used as standard.

Determination of Oxidative Stress Index (OSI)

OSI, an indicator of the degree of oxidative stress is the percentage ratio of the total plasma peroxide (µMol H_2O_2/L) to the total antioxidant activity (µmoL/L) (Benzie and Strain, 1996).

Determination of Uric Acid

Principle: Uric acid is converted by uricase to allantoin and hydrogen peroxides, which underthe catalytic influence of peroxidase, oxidizes 3,5 – dichloro – 2 – hydroxybenzene sulfonic acid and 4-aminophenazone to form a red violet quininemine compound (Fossati, 1980).

Determination of Total Antioxidant Activity (TAA)

Principle: A standardized solution of Fe-EDTA complex reacts with hydrogen peroxides by a fenton-type reaction, leading to the formation of hydroxyl radicals (OH). These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substance (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development defined as antioxidant activity (Harma et al., 2003).

Determination of Glutathione Peroxidase

Principle: Glutathione peroxide (GPx) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340nm is measured.

Statistical Analysis

Data were analyzed using Statistical Package of Science and Social Science (SPSS) software version 15 and Epin info. Independent Students' 't' test was used to compare groups. Pearson's correlation coefficient determination was performed to evaluate the degree of association. Values were expressed as means ± standard deviation (SD). Probability values of less or equal to 0.05 were accepted to be significant ($P \leq 0.05$).

RESULTS

The mean values of lead, zinc, iron, selenium, vitamin c and uric acid of the auto painters and the control subject are presented in Table 1. There were significant decreases in the mean levels of zinc, iron, selenium, vitamin C and uric acid of the auto painters compared with the control subjects ($p < 0.05$). A highly significant increase in lead was observed in the auto painters when compared with the control subjects ($p < 0.0001$).

The mean values of glutathione peroxidase, catalase, superoxide dismutase, total antioxidant activity, total plasma peroxide, and oxidative stress index of the auto painters compared with the control

Table 1. Concentration of Pb, Zn, Fe, Se, vitamin C and Uric acid in auto painters and controls.

Variables	Auto Painters (n=50)	Control (n=50)	t-value	p-value
Pb (µg/dL)	28.92±16.30	8.75 ±2.39	5.595	0.0001*
Zn (µg/dL)	70.56±11.26	86.66 ±14.60	-2.212	0.047*
Fe (µg/dL)	84.26 ±12.44	91.98 ±8.87	-3.572	0.001*
Se (µg/dL)	40.40 ±5.91	47.72 ±5.25	-6.550	0.0001*
Vitamin C (mg/dL)	1.58 ±0.41	2.01 ±0.39	-5.258	0.0001*
Uric acid (mg/dL)	4.03 ±0.88	4.94 ±0.74	-3.134	0.012*

Results are expressed as mean ± SD
*= Significantly different from controls
n = number of subjects

Table 2. Concentrations of markers of oxidative metabolic changes in auto painters and controls.

Variables	Auto Painters (n = 50)	Control (n = 50)	t-value	p-value
GPx (iu/L)	105.14 ±22.11	130.12±10.14	-7.263	0.0001*
CAT (µmoL/L)	68.06 ± 11.51	79.50± 14.86	-4.303	0.0001*
SOD u/mL	107.60±15.69	152.84± 14.70	-14.87	0.0001*
TAA mmoL/L	1.86 ±0.41	2.17 ±0.40	-3.806	0.0001*
TPP µmoL/L	27.49± 6,94	15.40± 2.27	11.67	0.0001*
OSI	1.606± 0.59	0.74± 0.18	9.95	0.0001*

Results are expressed as mean ± SD
* = Significantly different from controls
n = number of subjects

subjects are illustrated in Table 2. The antioxidants (GPx, CAT, SOD, and TAA) were significantly reduced in auto painters when compared with control subjects($p<0.0001$). The TPP and the oxidative stress index (OSI) were significantly raised in auto painters. ($p < 0.0001$). The mean values of lead, zinc, iron, selenium, vitamin C and uric acid of the battery chargers and the control subject are presented in Table 3. There were significant decreases in the mean levels of zinc, iron, selenium, vitamin C and uric acid of the battery chargers compared with the control subjects ($p < 0.01$). A significant increase in lead was observed in the battery chargers when compared with the control subjects ($p < 0.0001$).

The mean values of glutathione peroxidase, catalase, superoxide dismutase, total antioxidant activity, total plasma peroxide, and oxidative stress index of the battery chargers compared with the control subjects are illustrated in Table 4. The antioxidants (GPx, CAT, SOD, and TAA) were significantly reduced in battery chargers when compared with control subjects. ($p < 0.0001$). The TPP and the oxidative stress index were significantly raised among battery chargers. ($p < 0.0001$).

Levels of Association between lead and oxidative stress indices and micronutrients in battery chargers are shown in Table 5. Inverse correlations were shown between lead and micronutrients. In contrast positive correlations were shown between lead and TPP and lead and OSI. The findings show that lead

Table 3. Concentrations of lead, zinc, iron, selenium, vitamin C and uric acid in battery chargers and controls.

Variables	Battery Chargers (n=50)	Controls (n=50)	t-value	p-value
Pb (µg/dL)	59.91± 25,42	8.75± 2.39	8.414	0.0001*
Zn (µg/dL)	62.72± 10.76	86.66± 14.60	9.334	0.0001*
Fe (µg/dL)	51.54± 9.99	91.98± 8.87	-21.400	0.0001*
Se (µg/L)	28.88 ±8.47	47.72± 5.25	-13.372	0.0001*
Vitamin C (mg/dL)	0.99± 0,28	2.01± 0.39	-14.727	0.0001*
Uric acid (mg/dL)	3.29± 0.71	4.97± 0.74	-10.192	0.001*

Results are expressed as mean ± SD
* = significantly different from controls
n = number of subjects

Table 4. Concentrations of markers of oxidative metabolic changes in battery chargers and controls.

Variables	Battery chargers (n=50)	Controls (n=50)	t-value	p-value
GPx(iu/L)	78.12 ±13.29	130.12±10.14	21.998	0.0001*
CAT (µmoL/L)	55.72 ±11.91	79.50±14.86	8.82	0.0001*
SOD (U/mL)	96.66± 17.91	152.84± 14.70	17.144	0.0001*
TAA (µmoL/L)	1.27± 0.32	2.17± 0.40	12.320	0.0001*
TPP (µmol/L)	36.46±6.78	15.40±2.27	10.236	0.0001*
OSI	3.05± 1.16	0.74± 0.18	13.829	0.0001*

Results are expressed as mean ± SD
* =significantly different from controls
n = number of subjects

Table 5. Levels of correlation between lead and oxidative stress indices, trace metals in battery chargers.

Variables	Lead (r)	p- value
TPP	0.692	(0.0001)**
OSI	0.829	(0.0001)**
Zn	-0.840	(0.0001)**
Iron	-0.921	(0.0001)**
Se	-0.693	(0.0001)**

** Correlation is significant at 0.01 levels.

toxicity can suppress micronutrient activity and also induce oxidative stress in battery chargers.

Levels of Association between lead and oxidative stress indices and trace metals in auto painters are shown in Table 6. Inverse correlations were shown between lead and trace metals. In contrast positive correlations were shown between lead and TPP and lead and OSI. The findings show that lead toxicity can suppress micronutrient activity and also induce oxidative stress in battery chargers.

DISCUSSION

Significantly lower levels of micronutrients were corroborated in the work of Wasowicz et al., (2001) who recorded reduced serum zinc and selenium

Table 6. Correlation of lead and oxidative stress indices, and trace metals among auto painters.

Variables	Lead (r)	p- value
TPP	0.793	(0.0001)**
OSI	0.931	(0.0001)**
Zinc	-0.901	(0.0001)**
Iron	-0.905	(0.0001)**
Se	-0.796	(0.0001)**

** Correlation is significant at 0.01 levels

among workers exposed to lead and cadmium. Also significantly reduced levels of antioxidant enzymes in auto painters and battery chargers agreed with the work of Khaki et al., (2010) who introduced lead acetate (1 %) into rats and recorded reduced levels of superoxide dismutase and catalase compared with the controls as antioxidant systems are trace metal dependent. Adequate plasma levels of iron and zinc are necessary for the synthesis of superoxide dismutase; iron is very crucial in the normal synthesis of catalase while selenium is an integral part of glutathione peroxidase (Baker et al., 1993). The deficiencies of these essential trace metals have been reported as a factor that may cause deficiencies of superoxide dismutase, glutathione peroxidase and catalase (Wasowicz et al., 2001). Lead toxicity among the workers could have been the cause of lower levels of the micronutrients observed because of the interference of lead with these essential metals. The indirect effect of lead to SOD, GPx and CAT therefore is because these antioxidant enzymes depend on various essential trace metals for proper molecular structure and activity. For example, selenium is a co-factor of glutathione peroxidase, a cyto-antioxidant enzyme enhancing the availability of GSH, which is one of the most abundant intrinsic antioxidants that help in preventing lipid peroxidation and resultant oxidative stress and cell damage. It shows therefore that lower levels of selenium could lead to oxidative stress via this pathway.

The relative importance and interaction between different antioxidants are very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another. The action of one antioxidant may therefore depend on the proper function of the other members of the antioxidant system. The amount of protection provided by one antioxidant system will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered and the status of the antioxidants with which it interacts (Vertuani et al., 2004). In general, antioxidants systems either prevent these ROS or free radicals from being formed or remove them before they can damage vital components of the cells (Devasagayam et al., 2004). Continuous neutralization of high free radical load by the antioxidant system could lead to the exhaustion of the antioxidants and this could have caused the significant decreased levels of total antioxidant activity, vitamin C and uric acid in this study.

The ratio of the total plasma peroxide (TPP) levels to the total antioxidant activity (TAA) gave the oxidative stress index (OSI), an indicator of the degree of oxidative stress (Harma et al., 2003). The OSI was employed to emphasize the differences in oxidative stress and antioxidants values between the subject group and the control group. The increased TPP and OSI were consistent with the works of Alessandra et al., (2009) who observed similar variations. An inverse linear correlation between lead and trace metals observed in our study showed that lead suppresses the micronutrient activity in lead workers. This study showed that auto painters and battery chargers have higher levels of oxidative stress and diminished levels of antioxidants and trace metals. Studies have shown that lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS), reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzymes activities and increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition (Guere and Ercal, 2002).

CONCLUSION

An elevated blood lead level among Nigerians unexposed to lead was observed in this study. This

suggests that an average member of the public may be living with a blood lead burden of about 8.75 µg/dl or more. It is course for concern because of the harmful nature of lead at all levels. The exposure to lead fumes and dusts in the workplace of battery chargers and auto painters significantly increased the blood lead levels of the workers and may have suppressed the micronutrient and antioxidant status to induce oxidative stress during exposure.

RECOMMENDATIONS

It is recommended that strict regulations limiting the amount of lead in paints and petroleum products must be adhered to. There should be close medical supervision of the work place and constant public enlightenment of the lead workers on the use of personal protective equipment like safety goggles, and face shield for the eyes, coveralls, boots and protective clothing to prevent penetration through skin.

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Conflict of Interest

All authors of this article report no conflict of interest throughout the work.

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